REMARKS

I) CLAIM OBJECTIONS

The Examiner has objected to the claims on several grounds. Applicants have amended the claims[1] to address each of these objections and respectfully request the objections be withdrawn.

A) Deletion of the phrase "selected from the group consisting of" was objected to in claim 32(b). This phrase has been added to claim 32(b), and to claims 1(e) and 23(e).

B) The Examiner has requested that the term "solid tumor stem cells" be inserted before "claim 23" in claim 194. This phrase has been added to claim 194 as requested.

C) Claim 205 is objected to for use of the incorrect amendment format. Claim 205 has been amended in the present amendment, using the proper format: "previously presented."

D) Claims 34, 35, 38, 40, 188, 204, 210 were objected to as being dependent upon a rejected base claim although no basis for the rejection of claim 32 is provided in the Office Action.

II. CLAIM REJECTION

In the Office Action dated December 1, 2004 the Examiner has made a number of rejections. The currently pending rejections are:

1) Claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 are rejected under 35 U.S.C. 102(b) as anticipated by *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn").

[1] Applicants do not necessarily agree that the claims need to be amended, but do so to further the prosecution of the present application and reserve the right to pursue claims having the original language (or similar language) in the future.

2) Claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 are rejected under 35 U.S.C. 102(b) as anticipated by *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli").

3) Claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli"), and as evidenced by *Janeway, Jr. et al.* ("Janeway") (Immunobiology, 1999) and *Thampoe et al.* ("Thampoe") (Arch Biochem Biophys. 1988;267:342-52).

4) Claims 20 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli"), as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of *Salmon et al.* ("Salmon") (New England J Med 1978;298:1321-7).

5) Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli"), as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of *Salmon et al.* ("Salmon") (New England J Med 1978;298:1321-7).

6) Claims 8-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli"), as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of *Nierodzik et al.* ("Nierodzik") (Blood 1998;92:3694-3700).

7) Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli"), as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of *Bromberg et al.* ("Bromberg") (PNAS 1995;92:8205-9).

Applicants note that certain of the claims (32, 200, 202, 203, 206, and 208) are not part of the recited rejections or objections, and yet are not designated as allowed in the Office

Action. During an Interview with the Examiner, it was noted that these claims are allowable in view of the amendments made herein, rendering the need to address these claims moot.

Applicants believe the pending claims are not taught by the cited documents and are not obvious. Therefore, claims 1, 4, 6, 8-14, 18-23, 28-30, 32, 34, 35, 38, 40, 188, 194, and 199-223 should be passed into allowance.

Applicants wish to thank the Examiner for taking the time to conduct an in-person interview.

A. The Cited References Do Not Anticipate the Claims

Before addressing each claim rejection, Applicants would like to discuss the invention and the clarifying amendment made to claims 1 and 23. Dr. Clarke and co-inventors discovered that a small percentage of cells within a solid tumor behave like stem cells, that is they are tumorigenic stem cells – they give rise to additional tumorigenic stem cells ("self-renew") and they differentiate by giving rise to non-tumorigenic tumor cells (cancer cells unable to give rise to a tumor). Said another way it is the solid tumor stem cell that gives rise to new tumors that could be serially transplanted and further form new tumors. This invention for the first time discovered that a distinct small subset of cells from a solid tumor have properties of "stem cells" in that they, for example, proliferate extensively and indefinitely and give rise to additional solid tumor stem cells, that is they self-renew. Dr. Clarke and co-inventors discovered the small subpopulation of solid tumor stem cells and provided the means for identifying those solid tumor stem cells and distinguishing them from the non-tumorigenic tumor cells. There are two aspects to Dr. Clarke's means for identifying the tumor stem cells: first identifying a pattern of surface markers to enable one to prospectively identify the solid tumor stem cells; and, second using a functional tumorigenic assay to distinguish between the tumorigenic capacity of the isolated solid tumor stem cells and the non-tumorigenic capacity of the solid tumor cells. The examples in the patent application establish for the first time that heterogeneous populations of cells in solid tumors, for example, breast cancer or ovarian cancer, contain a distinct tumorigenic solid tumor stem cell population as well as a much larger solid tumor cell population that lacks the tumorigenic capacity. This invention for the first time identified and enabled the isolated

or enriched population of solid tumor stem cells that are tumorigenic as well as providing the ordinarily skilled artisan the means and methods for isolating and enriching for that population of solid tumor stem cells. Experiments conducted during the development of the present invention conclusively demonstrated that solid tumors, such as breast cancers: are functionally heterogeneous; contain a very small subpopulation of solid tumor stem cells that are capable of establishing a cancer after transplantation through self-renewal (i.e. make other solid tumor stem cells) as evidenced by their tumorigenic capacity on serial transplantation and differentiation by giving rise to solid tumor cells – the majority of cells in a solid tumor that are non-tumorigenic on serial transplantation. The amendment to claims 1 and 23 clarifies that, in addition to the pattern of surface markers, the claimed isolated population of solid tumor stem cells are tumorigenic (element (a)(i) of claim 1 and element (b) of claim 23) while the claimed population of solid tumor cells are non-tumorigenic (at the end of claims 1 and 23).

It is noteworthy that accompanying Dr. Clarke's publication of his cancer stem cell discovery in PNAS USA 100(7), 3983-3988 (2003) was a commentary by John Dick entitled, "Breast Cancer Stem Cells Revealed." (PNAS USA 100(7), 3547-3549, 2003)

This paper points out the fundamental nature of Dr. Clarke's discovery first by setting out the problem, "...a fundamental problem in cancer research is the identification of the cell type capable of sustaining the growth of the neoplastic clone.... What is less clear for most cancers is which cells within the tumor clone possess tumor-initiating cell (T-IC) function and are capable of maintaining tumor growth." Id. At 3983. [T-IC is Dick's name for the solid tumor stem cells identified by Dr. Clarke et al.] Dick describes Dr. Clarke's paper as "...provid[ing] a major step forward with the identification of human breast cancer initiating cells (BrCa-IC)....First a reliable xenograft assay was developed...that provides a reliable and sensitive *in vivo* T-IC assay for human breast cancer.... This study (referring to Dr. Clarke's work) demonstrates the crucial importance of combining cell sorting with functional assays.... Collectively, these results conclusively demonstrate that breast cancer is functionally heterogeneous and that a rare BrCA-IC is the only cell type capable of establishing human breast cancer after transplant.... Id. At 3983. The commentary goes on to point out "...another important property of BrCA-IC [is] the ability to reestablish tumor heterogeneity after

transplantation.... Importantly, secondary transplant experiments indicated that [tumor stem cells] were the only fraction with the capacity for tumor initiation on serial transplant. These data provide strong support for the idea that on cell division the BrCa-IC can self-renew (i.e., make other BrCa-IC) as well as make progeny that acquire maturation markers and lose the ability to initiate tumor growth. Id. at 3548.

During the interview with the Examiner, it was agreed the claims would be patentable upon the recitation in claims 1 and 23 that the solid tumor stem cells do not express CD24 or express low levels of CD24. These amendments have been made and Applicants accordingly believe the claims are patentable. As discussed below, the cited references do not teach or suggest this element as well as other elements of the claims.

In the Office Action of December 1, 2003, claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, and 209 were rejected under 35 U.S.C. 102(b) as being anticipated by Hathorn or Piselli, and as evidenced by Janeway and Thampoe. None of the cited references teach or suggest the claimed isolated or enriched population of solid tumor stem cells that are tumorigenic, in contrast to solid tumor cells that are not tumorigenic, nor do those cited references provide the means and methods for isolating or enriching for that isolated or enriched population of solid tumor stem cells.

Hathorn et al. is cited as teaching a population of tumor cells isolated by fluorescence-activate cell sorter (FACS) with an antibody that recognizes CD44. Hathorn is then combined with Janeway et al to allegedly teach that CD44 cells would lack detectable levels of expression of the recited Lineage CD markers. Applicants respectfully traverse.

The Hathorn et al. study is directed to evaluation of the local production of cytokines IL-2 and/or IFN-α on the biologic properties of renal cell cancer (particularly certain intercellular adhesion molecules – ICAM-1 and CD44) that is associated with tumor invasion (page 1905, left column). Hathorn et al. modified the renal cell carcinoma cell line R11 by inserting various genes into the cells to produce certain cytokines locally and then evaluated by *in vitro* assays the effect on cell attachment and aggregation (assays described at page 1905, right column). Hathorn et al. observed that certain cytokine-producing R11 transfected cell lines showed lower efficiency in forming homotypic aggregation then did the control line suggesting a lower metastatic potential.

(Page 1910, right column first full paragraph.) The R11 cell line of Hathorn et al. is not the isolated or enriched population of solid tumor stem cells of the instant invention. Rather the R11 cell line is a cultured cell line and is a heterogeneous population of tumor cells. A series of papers indicate that many cancers are maintained by a hierarchical organization that includes slowly dividing stem cells, rapidly dividing transit amplifying cells (precursor cells) and differentiated cells. (See for example, Woodruff, 1983, BR. J. Cancer **47**, 589-594; Sell & Pierce, 1994, Lab. Invest. **70**, 6-22; and Reya et al., 2001 Nature **414**, 105-111.) In fact, Woodruff et al., cited abundant evidence that the "...proliferating cells of a primary malignant tumour at a particular time may be markedly heterogeneous in respect of such diverse properties as karyotyping... metastatic capacity as judged either by the development of spontaneous metastases... or by lung colony formation after i.v. injection of tumour cells... the presence of hormone receptors... sensitivity to cytotoxic drugs... expression of surface antigens...immunogenicity and/or responsiveness to the immune reaction of the host... tumorigenic capacity on transplantation... and morphology and growth rate in tissue culture.... (Woodruff et al., Ibid at 590). Malignant gliomas, for example, often contain both undifferentiated and differentiated cells and sometimes contain cells that express neuronal markers as well as cells that express glial markers, suggesting they may contain multipotent neural stem cell (NSC)-like cells. (Wolf et al, 1997, Acta Neuropathol. **94**, 302-320; Wharton et al. 1998, Neropathol. App. Nerobiol **24**, 302-308; and, Katsetos et al, 1998, J. Neuropathol.Exp. Neurol. **61,** 307-320.) Kondo et al., which is not prior art, have shown that many cancer cell lines in culture are heterogeneous populations of cells, even when those cancer cell lines have been maintained for many years. (Kondo et al., PNAS 2004, **101**(3), 781 – 786, second paragraph of the discussion.) In addition, Deschaseaux et al. showed that human bone marrow mesenchymal stem cells can be isolated from human bone marrow and then maintained in *in vitro* culture. (Deschaseaux et al., Brit. J. Haematology 2003, **122**, 506-517.) Significantly, Deschaseaux et al. observed that fresh mesenchymal stem cells obtained from human bone marrow are $CD45^+$ and became $CD45^-$ when cultured. The changes were induced by cell culture. (Deschaseaux et al. at 514.) Thus, the markers expressed in cultured cells do not necessarily correlate to the marker expressed by the corresponding cells in different

environments (e.g., in vivo). It is for those reasons that Hathorn et al.'s observation of local tumor formation following injection of the R11 cell line is not tumor formation from an isolated or enriched population of solid tumor stem cells. There is no evidence in Hathorn et al. that the R11 cultured cell line is anything other than a heterogeneous population (as would be predicted in light of the work cited above) nor is there any disclosure or suggestion in Hathorn et al. that the observed tumor growth is attributable to a subpopulation of cells within the R11 cell line. Furthermore, Hathorn et al. neither disclose nor suggest that the R11 cell line is an isolated or enriched population of solid tumor stem cells that are tumorigenic. Finally, Hathorn is a cultured cell line and as such those cells are likely different than fresh renal carcinoma cells and as such say nothing about whether there are solid tumor stem cells that are tumorigenic. Id at 514. Because there is no evidentiary basis demonstrating that the cultured cells of Hathorn et al. are isolated or enriched tumorigenic solid tumor stem cells as claimed, the rejection must be withdrawn.

In summary, Hathorn does not set forth each and every element of claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, and 209. In specific:

- Hathorn does not teach an isolated population of solid tumor stem cells that comprises at least 75% solid tumor stem cells. (Claim 1).
- Hathorn does not teach a solid tumor stem cell population that is enriched at least 2-fold. (Claim 23).
- Hathorn does not teach tumorigenic solid tumor stem cells that express CD44 and do not express detectable levels of one or more LINEAGE markers selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b and do not express CD24 or express low levels of CD24. (Claim 1 and 23).
- Hathorn does not teach the isolated population of solid tumor stem cells isolated by the method of claim 40. (Claim 199). Moreover, Hathorn does not teach the method of claim 40, or the method of claim 32.

The Examiner cites Piselli et al. as teaching a population of tumor cells isolated by FACS with an antibody that recognizes CD44 wherein the tumor is adenocarcinoma (solid tumor of epithelial origin). Piselli et al. is again combined with Janeway et al. to

teach the CD44 cells would lack detectable levels of expression of the recited CD markers. Applicants respectfully traverse.

Piselli et al. was also a study focused on the role of cell surface markers in the metastatic process by comparing phenotypes expressed by cells of primary tumors and of metastatic lesions. (Piselli at page 826, upper left column.) Human pancreatic adenocarcinoma (HPC-4) cells were injected into the left legs of SCID mice and growth of tumors was observed. As shown in table I of Piselli (page 829) and in figure 3, page 828, the HPC-4 cells grown in culture and the cells injected into the SCID mice were in fact five populations of cells – those expressing CD44H, CD44v5, CD44v6, ICAM-1 and HSP60. In addition, Piselli like Hathorn is again a cultured population of adenocarcinoma cells and as explained above placing cells in culture induces phenotypic changes. As such, one cannot conclude that the properties of the HPC-4 cells are in fact the properties of fresh human pancreatic adenocarcinoma cells. Even assuming arguendo that Piselli's observations are properties of human pancreatic adenocarcinoma cells, Piselli et al. neither disclose nor suggest the small population of solid tumor stem cells within HPC-4 that gives rise to those primary tumors. Piselli et al neither disclose nor suggest the isolated or enriched population of solid tumor stem cells that are tumorigenic nor does Piselli et al. provide the means and methods for isolating the population of solid tumor stem cells.

In summary, Piselli does not set forth each and every element of claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, and 209. In specific:

- Piselli does not teach an isolated population of solid tumor stem cells that comprises at least 75% solid tumor stem cells. (Claim 1).
- Piselli does not teach a solid tumor stem cell population that is enriched at least 2-fold. (Claim 23).
- Piselli does not teach tumorigenic solid tumor stem cells that express CD44 and do not express detectable levels of one or more LINEAGE markers selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b and do not express CD24 or express low levels of CD2. (Claim 1 and 23).

- Piselli does not teach the isolated population of solid tumor stem cells isolated by the method of claim 40. (Claim 199). Moreover, Piselli does not teach the method of claim 40, or the method of claim 32.

Nor does the Examiner's addition of Janeway to Hathorn or Piselli cure these many defects. Janeway's table teaches that certain of the Lineage markers of the present invention are expressed on endothelial cells. Janeway does not teach that the Lineage markers of the present invention are not found on tumorigenic solid tumor stem cells, or on epithelial tumorigenic solid tumor stem cells, or on non-tumorigenic solid tumor cells, or on epithelial non-tumorigenic solid tumor cells. Janeway combined with Hathorn or Piselli, fails to suggest or disclose, an isolated population of solid tumor stem cells that are tumorigenic, an enriched population of solid tumor stem cells that are tumorigenic, and methods for isolating a population of solid tumor stem cells that are tumorigenic.

Thampoe et al. is cited as establishing that the ESA cell surface marker would be an inherent characteristic of renal carcinoma cells or adenocarcinoma cells. The Examiner argues that because the cited document's cancer cells had the common characteristics – CD44, lack of Lineage markers, epithelial origin and solid tumors – with the solid tumor stem cells of the instant invention – *"...they would have reasonably suggested the existence of the same or similar type of epithelial solid tumor stem cell...."* Finally, the Examiner argues that "...the burden is upon the applicant to prove that the cited document's products do not necessarily or inherently possess characteristics of the claimed product...." Applicants respectfully traverse.

None of the references cited to date disclose or suggest an isolated or enriched population of solid tumor stem cells that are tumorigenic. Thampoe teaches that ESA is present on epithelial cells and epithelial tumor cell lines. Thampoe does not teach that ESA is present, or absent, on solid tumor stem cells that are tumorigenic, nor does Thampoe teach the combination of CD44 positive and Lineage negative screening with or without ESA screening. It is only with the benefit of the teaching of the present invention that one looks back at experiments trying to elucidate the role of certain cell surface markers in cancer and tries to attribute to heterogeneous populations of cells the characteristics of the isolated solid tumor stem cell population of this invention. While it is theoretically possible that one or more of the populations of cells that comprise the

cultured R11 cell line or cultured HPC-4 cells may possess a CD44 surface marker, lack certain lineage markers and express ESA, none of the cited references identify the very small population of cells that is the isolated or enriched population of solid tumor stem cell that is tumorigenic of the present invention nor do those references provide the means and methods for isolating the solid tumor stem cell population that is tumorigenic. Nor do the cited references provide the means for using the cited cell surface markers for identifying the predominant cancer cell population that is non-tumorigenic. There is no laboratory comparison to be made as none of the cited documents disclose or suggest an isolated or enriched solid tumor stem cell population to compare to the solid tumor stem cell population of the instant invention. Hence, the Examiner's combination of Hathorn or Piselli with Janeway and/or Thampoe fails to disclose not just one, but many elements of the claimed invention including CD44 positive/Lineage negative solid tumor stem cells that are tumorigenic, an isolated population of solid tumor stem cells, an enriched population of solid tumor stem cells, and the means and methods for isolating a population of solid tumor stem cells that are tumorigenic and consequently an enriched population of solid tumor cells that are non-tumorigenic. Thus, the Examiner's combinations fail to teach each and every element of the presently claimed invention, and without more the Examiner is unable to sustain the rejection of the claims. In view of the above, the Applicants respectfully request that the rejection be withdrawn.

Claim 199 was rejected because the claimed method of isolating/enriching the solid tumor stem cells would not distinguish them over the tumor stem cells taught by the cited documents. Applicants respectfully traverse for the reasons set forth above in that none of the cited references, alone or in combination, either suggests or discloses the isolated or enriched population of tumorigenic solid tumor stem cells of the instant invention. The rejection cannot stand for several reasons. First, the Examiner fails to cite a reference disclosing the claimed solid tumor stem cells that are tumorigenic. Second, the cited documents' tumor cells of Hathorn or Piselli are cultured tumor cells that are likely to be very different than fresh tumor cells and importantly the tumor cells of Hathorn or Piselli are not the claimed isolated/enriched population of solid tumor stem cells that are tumorigenic. Third, contrary to the Examiner's assertion, because the isolated/enriched populations of tumorigenic solid tumor stem cells of claim 199 are

defined by their method of manufacture (*i.e. screening using certain selective markers such as* CD44 and Lineage markers) they are distinguished from the cited documents which fails to teach this method of manufacture. Hathorn does not teach the claimed isolated/enriched population of solid tumor stem cells that are tumorigenic. Hathorn does not teach the means or methods for isolating or enriching for the isolated solid tumor stem cell population that is tumorigenic. Piselli does not teach claimed isolated/enriched population of solid tumor stem cells that are tumorigenic. Piselli does not teach the means or methods for isolating or enriching for the isolated solid tumor stem cell population that is tumorigenic.

A product-by-process composition claim is patentable when the resulting composition derived from the method is not found in the cited art. This is unquestionably the case for the present claims. None of the documents cited by the Examiner teach or suggest the claimed isolated/enriched population of solid tumor stem cells that are tumorigenic isolated by the recited method. None of the methods of the cited art would or could result in the isolated or enriched populations of solid tumor stem cell produced by the claimed methods. By definition, the screening of a cell population based on a particular marker or series of markers will generate a different product than a second method that uses a different marker or series of markers. None of the Examiner's cited references teach or suggest the means or methods using the claimed series of marker(s). Thus, the product-by-process composition claims of the present invention are novel and non-obvious, and the rejection of claim 199 must be withdrawn.

In view of the above, Applicants request that the 35 U.S.C. 102(b) rejections be withdrawn.

B. The Claimed Subject Matter is Non-obvious

Claims 20 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al.* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli") as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of Salmon et al (New Eng J Med 1978;298:1321-7). Examiner indicates that Hathorn or Piselli do not teach to place tumor cells in the affixed substrate and/or to treat cells to reduce proliferation, Salmon

supplements the teaching of Hathorn or Piselli by illustrating that these are routines in the process of investigating a solid tumor stem cell. Accordingly it would have been obvious to modify the methods by simply substituting the ovarian cancer cells as taught by Salmon with the renal carcinoma or adenocarcinoma cells as taught by Hathorn or Piselli. The motivation would have been to modify the claimed invention because they allegedly are necessary steps for cancer investigation. Applicants respectfully traverse.

Salmon is of record and has been discussed before. For completeness, Salmon focuses on developing a tumor colony assay to measure drug sensitivity of human tumor cells to various anticancer drugs. Salmon collected samples from patients with malignant ovarian effusions and bone marrow cells from patients with myeloma. Cells were plated and colony formation was measured. The ovarian tumor samples of Salmon were collected, centrifuged, washed in buffer and filtered through gauze. (Salmon Materials and Methods at 1322). In light of the work of Post and Grieg and the other papers cited above, the tumor cells of Salmon that are tested for colony formation are, at best, a heterogeneous population of tumor cells. Regardless of what Salmon calls the cells Salmon's cells contain multiple populations of cells obtained from ovarian tumors and are not the isolated or enriched solid tumor stem cells that are tumorigenic of the present invention. Furthermore, even assuming arguendo that the cells of Salmon are solid tumor stem cells[2], there is no disclosure in Salmon that establishes that colony formation *in vitro* is a predictor of tumor formation in vivo. All that Salmon discloses is tumor cells comprising multiple populations of tumor cells that give rise to colony formation *in vitro*. Since non-tumorigenic cells can form colonies, the clonogenic assay of Salmon does not distinguish tumorigenic from non-tumorigenic cancer cells. For these reasons, Salmon neither discloses nor suggests the claimed isolated or enriched solid tumor stem cells that are tumorigenic nor the means and methods for isolating or enriching the solid tumor stem cells that are tumorigenic. For the reasons discussed above none of the references alone or in combination either disclose or suggest the isolated or enriched population of solid tumor stem cells that are tumorigenic applied to a substrate. Finally, there would be no reason to apply the cells of Hathorn or Piselli (assuming arguendo they were the solid tumor stem cells of this invention) to a fixed substrate in the manner of Salmon because

[2] For the reasons discussed above, Applicants assert that they are not.

whether or not colonies are formed are irrelevant to the claimed invention. Furthermore, a *prima facie* case of obviousness requires the citation of references that a) disclose all the elements of the claimed invention, b) suggest or motivate one of ordinary skill in the art to combine the claim elements to yield the claimed invention, and c) provide a reasonable expectation of success should the claimed combination be carried out. Because not one, but each of the three elements of a *prima facie* case of obviousness is lacking, the Applicant respectfully requests that the rejections under 35 USC §103(a) for alleged obviousness be withdrawn.

Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al.* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli") as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of Salmon et al (New Eng J Med 1978;298:1321-7). Claim 22 is drawn to solid tumor stem cells treated to increase proliferation. Again Salmon is cited as supplementing the teaching of Hathorn or Piselli by illustrating that it is routine and necessary in the process of investigating a solid tumor cell to promote cell growth. For the reasons discussed above none of the references alone or in combination either disclose or suggest the claimed isolated or enriched population of solid tumor stem cells that are tumorigenic.

Claims 8-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al.* (Cancer 1994,74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli") as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of Nierodzik et al (Blood 1998;92:3694-3700). Claims 8 -13 are drawn to an isolated solid tumor stem cell containing a polynucleotide vector. (Applicants respectfully note that claim 8 is drawn to solid tumor stem cells that are breast cancer stem cells or ovarian cancer stem cells. Claim 9 is drawn to an isolated solid tumor stem cell containing a polynucleotide vector. It is assumed the rejection should be drawn to claims 9 – 13.) Nierodzik is seen as supplementing the teaching of Hathorn or Piselli by establishing that it is well known in the art to use tumor cells as a nucleic acid carrier encoding therapeutic agents. Applicants respectfully traverse.

For all the reasons of record none of the references alone or in combination either disclose or suggest the claimed isolated or enriched population of solid tumor stem cells

that are tumorigenic or the means and methods for isolating or enriching for the claimed solid tumor stem cells.

Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Hathorn et al.* (Cancer 1994;74:1904-11) ("Hathorn") or *Piselli et al.* (Anticancer Res 2000;20:825-832) ("Piselli") as applied to claims 1, 4, 6, 18, 19, 23, 28-30, 199, 201, 203, 207, 209 above, and further in view of Bromberg et al (PNAS 1995;92:8205-9). Applicants respectfully traverse.

Claim 14 is drawn to the recombinant polynucleotide being integrated into a chromosome of the solid tumor stem cell. Once again for all the reasons stated above none of the references alone or in combination teach or suggest isolated/enriched solid tumor stem cells that are tumorigenic comprising a recombinant polynucleotide integrated into the chromosome of the solid tumor stem cell.

CONCLUSION

It is respectfully submitted that Applicants' claims as amended should be passed into allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application Applicants encourage the Examiner to call the undersigned collect at (608) 218-6900.

Dated: 1/25/05

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